

Structural Elucidation of Newly Synthesized Potentially Active Binuclear Schiff Base Cu(II), Ni(II), Co(II) and Mn(II) Complexes Using Physicochemical Methods

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Abstract

Condensation of Benzene-1,4-dicarbaldenhyde and 2,6 diaminopyridine in 1:2 molar ratio was carried out and this condensed ligand was further followed by condensation with 5-bromo-2-hydroxybenzaldehyde forming a new symmetrical Schiff base ligand, H₂L with two N₃O potentially active sites. Then a new series of homo-binuclear Cu(II), Ni(II), Co(II) and Mn(II) complexes have been synthesized. The binding manner of the ligand to the metal, composition and geometry of the metal complexes were examined by various physicochemical methods like elemental analysis, conductivity measurements, magnetic moments, IR, ¹H-NMR, CV, ESR and electronic spectral studies. It has been originated that the Schiff base ligand with Cu(II), Ni(II), Co(II) and Mn(II) ions form binuclear complexes with general formula [M₂LY₂]Z₂ where Y=2, 2' bipyridyl and Z = acetate ion. The conductivity data confirm the electrolytic nature of the complexes. In all the complexes, the Schiff base, H₂L, ligand acts as dibasic with two N₃O tetradentate sites and coordinate with two metal ions to form binuclear complexes after the deprotonation of the hydrogen atoms of the phenolic groups in all the complexes via (C=N) of pyridyl nitrogen, (C=O) and the new azomethine (C=N) groups. UV-Vis spectra, ESR and magnetic moments have suggested octahedral stereochemistry for Cu(II), Ni(II), Co(II) and Mn(II) complexes. The cyclic voltammetric studies of these complexes in N,N-dimethylformamide indicate the structural changes during the course of redox reaction and quasi-reversible nature of the Schiff base binuclear complexes. The interaction of the complexes with calf thymus DNA (CT-DNA) has been investigated by UV absorption and viscosity methods, and the mode of CT-DNA binding to the complexes has been explored. The pUC18 DNA cleavage study was monitored by gel electrophoresis method. From this study, it was found that the homo-binuclear Schiff base metal complex cleaves pUC18 DNA in presence of the oxidant H₂O₂. The Schiff base ligand and their binuclear metal complexes were also evaluated for their antibacterial activity and was found to be energetic and inhibits the bacterial growth of *Bacillus subtilis*, *Staphylococcus aureus* (as Gram-positive bacteria) and *Klebsilla pneumonia* and *Escherichia coli* (as Gram-negative bacteria).

Keywords: Benzene-1,4-dicarbaldenhyde, N₃O donor sites, binuclear Schiff base complexes, octahedral geometry, H₂L type ligand.

Abbreviations: pUC: Plasmid University of California; DNA: Deoxyribose nucleic acid; SC: Super coiled; NC: Nicked circular; EDTA: Ethylene diaminetetraacetic acid; L: Ligand; M: Metal; Mpt: Melting point; nm: nanometer(s).

1 INTRODUCTION

The developments in field of bioinorganic chemistry have increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species (1-5). A rational control of the nuclearity of transition metal complexes is important to design systems with the desired properties, as some of these applications require the presence of more than one metal centre in the particular complex. Indeed, binuclear complexes may have very different reactivity than mononuclear counterparts, thereby enabling transformations inaccessible to single metal ions (6).

In addition to their interesting properties, DNA cleavage activity of transition metal complexes under physiological conditions has gained considerable current interest for varied applications of such complexes in nucleic acids chemistry (7). Some kind of metal complexes interacted with DNA could induce the breakage of DNA strands by covalent binding, electrostatic interactions, groove binding or by intercalation methods. In the case of cancer genes, after DNA strands are cleaved, the DNA double strands break. The replication ability of cancer gene is destroyed. The Compounds showing the properties of effective binding as

well as cleaving double stranded DNA under physiological conditions are of great importance since these could be used as diagnostic agents in medicinal and genomic research. Their ability of the individual metal ions to have quite distinct roles in the functioning of the metalloenzymes concerned has led to a search for symmetrical binucleating ligands, and also gaining insight into the pathways of electron transfers in biological systems.

In continuation of our series of investigations, we attempted to widen the scope of derivatization by providing more flexibility through Schiff base formation with Benzene-1,4-dicarbaldenhyde, 2,6 diaminopyridine and 5-bromo-2-hydroxybenzaldehyde and finally complexation with metal ions to form potentially active binuclear Schiff base complexes. The Schiff base structure affords a greater choice and flexibility, and complexation with a metal ion adds to the stability and versatility of the compounds.

2 Experimental section

All the chemicals were of reagent grade and the solvents were distilled before use according to the standard procedure.

2.1 Physical measurements

Elemental analysis (C, H and N) was obtained using Perkin Elmer elemental analyzer. The molar conductance of the complexes in DMF (10^{-3}) solution was measured at $27 \pm 3^\circ\text{C}$ with an Elico model conductivity meter. Infrared spectra were recorded on the Thermo Nicolet, Avatar 370 model FT-IR spectrometer using KBr disc in the range of $4000-400\text{ cm}^{-1}$. Electronic spectra were recorded at 300 K on a Perkin-Elmer Lambda 40 (UV-Vis) spectrometer using DMF in the range 200-800 nm. NMR signals were obtained from Bruker Advance III, 400MHz model spectrometer. EPR spectra of compounds were recorded on an E-112 ESR Spectrometer with X-band microwave frequency (9.5 GHz). The Redox nature of the complex in DMF was measured using CHI760 Electrochemical Analyzer by employing a platinum electrode as working electrode, Ag/AgCl as a reference electrode and platinum wire as auxiliary electrode. The working media consist of DMF containing 0.1M tetrabutyl ammonium perchloride (TBAP) as supporting electrolyte. TG studies were carried out in the range between $0-1000^\circ\text{C}$ using a NETZSCH model thermal analyzer.

2.2.1 Synthesis of Schiff base ligand

The Schiff base ligand was synthesized by adding Benzene-1,4-dicarbaldehyde (1 mM) in 20 ml of ethanol and 2,6 diaminopyridine (2 mM) in 20 ml ethanol slowly with constant stirring followed by the addition of 5-bromo-2-hydroxybenzaldehyde (2 mM). The mixture was refluxed for 3 hrs. Then solution of the ligand was kept for slow evaporation and coloured precipitate was collected and dried in air.

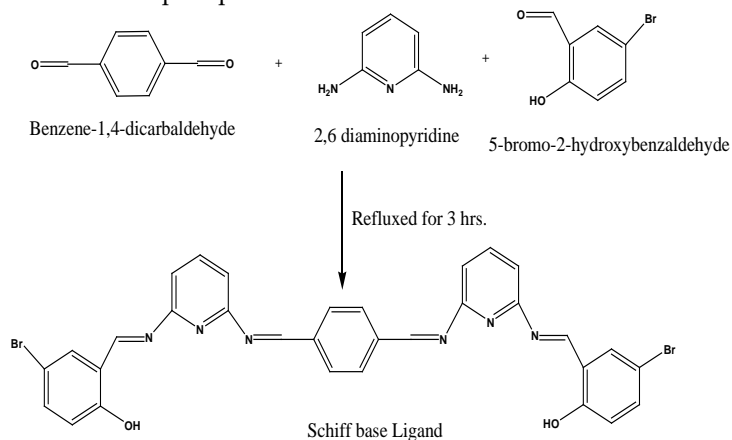
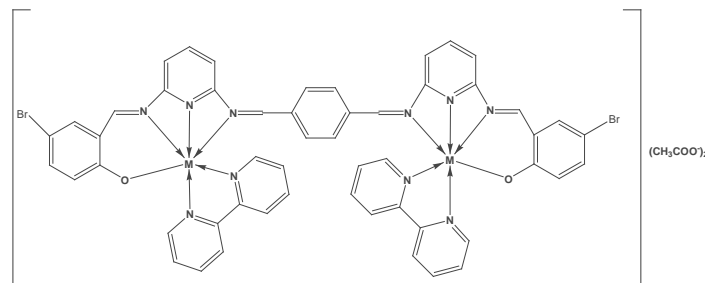


Fig.1 Synthesis of Schiff base ligand (H_2L)

2.2.2 Synthesis of binuclear Schiff base Metal complexes

The ethanolic solution of synthesized Ligand (H_2L) (1 mM) was added dropwise stirring to an ethanolic solution of the metal salt (2 mM) with constant stirring, followed by the

addition of 2,2'-bipyridyl and the mixture was boiled under reflux for 5 hrs. Then, the volume of the reaction mixture was reduced to 10 ml by evaporation. The precipitated complexes were filtered off, washed with ethanol and then dried in *vacuo*.



M= Cu(II), Co(II), Ni(II) & Mn(II)

Fig. 2 Synthesis of Binuclear Schiff base Metal complexes

2.3 Antibacterial activity

The *in vitro* antibacterial screening effects of the synthesized compounds were tested against four bacterial strains namely, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsilla pneumonia* by disk diffusion method, using nutrient agar medium. The solution with different concentration (25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 75 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$) was prepared by dissolving the compounds in DMF. The well was made on agar medium, inoculated with microorganisms. To this well, the test solution was added and the Petri dishes were incubated for 24 hrs. During this period, the test solutions are diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration was noted and measured in mm. Results were compared with standards namely *streptomycin* against bacteria.

2.4 Methodology for DNA binding analysis

2.4.1 Using viscosity measurement

Viscosity measurements were carried out using an Oswald micro viscometer, maintained at constant temperature (37°C) in a thermostat. The DNA concentration was kept constant in all samples, but the complex concentration was increased each time (from 20 to 100 μM). Mixing of the solution was achieved by bubbling the nitrogen gas through viscometer. The mixture was left for 10 min at 37°C after addition of each aliquot of complex. The flow time was measured with a digital stopwatch. The experiment was repeated in triplicate to get the concurrent values. Data are presented as Relative viscosities (η/η_0)^{1/3} versus the ratio $[\text{complex}]/[\text{DNA}] = R$, where η and η_0 are the specific viscosity of DNA in the presence and absence of the complex respectively.

2.4.2 Using electronic absorption spectroscopy

Titration with electronic absorption spectroscopy is an effective method to investigate the binding mode of DNA with metal complexes. The UV-Visible experiments were conducted by adding solution of DNA at different concentrations (0–350 μM) to the samples containing 3 mM copper complexes. Absorption spectra were recorded in visible region within the range of 250–600 nm. Samples were equilibrated before recording each spectrum.

2.5 Methodology for DNA cleavage analysis

2.5.1 Using Gel Electrophoresis

The cleavage of pUC18DNA (Fig. 9) in the presence of H_2O_2 has been studied by gel electrophoresis (8) using supercoiled (SC) pUC18DNA in 40 μM , 50 μM metal complexes and 50 μM H_2O_2 in Tris-HCl buffer (pH 7.2). In the present study, the pUC18DNA gel electrophoresis experiment was conducted at 37°C using our synthesized complexes in the presence of H_2O_2 as an oxidant. After incubation, the samples

were electrophoresed for 2 hours at 50 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 $\mu\text{g}/\text{cm}^3$ ethidium bromide for 30 min prior to being photographed under UV light at 360 nm. The conversion of complex to supercoiled DNA (Form I), to nicked circular form (Form II) or linear form (Form III) determines the cleavage efficiency. All the experiments were performed at room temperature.

3 Results and discussion

The compounds were analyzed for C, H, N while the metal contents in the complexes were estimated by standard methods. The isolated binuclear Schiff base metal complexes have general composition $[\text{M}_2\text{LY}_2]\text{Z}_2$ where M is Cu(II), Co(II), Ni(II) & Mn(II), Y=2,2'-bipyridyl and Z = acetate ion. The complexes are intense colored and thermally stable at room temperature, non-hygroscopic, insoluble in water, highly soluble in DMF and DMSO.

Table 1 Analytical data of the Schiff base ligand and its binuclear metal complexes

Compounds	Molecular Formula	color	M. Wt	Yield %	M Pt ($^\circ$)	% of Nitrogen		% of Metal	
						Cal	Exp	Cal	Exp
L ₁	C ₃₂ H ₂₂ Br ₂ N ₆ O ₂	orange	682.37	80	142	12.31	12.29	-	-
[Cu ₂ L ₁ X ₂]Y ₂	C ₅₆ H ₄₂ Br ₂ Cu ₂ N ₁₀ O ₆	Dark green	1237.9	75	>200	11.31	11.30	10.27	10.24
[Co ₂ L ₁ X ₂]Y ₂	C ₅₆ H ₄₂ Br ₂ Co ₂ N ₁₀ O ₆	Brown	1228.7	70	>200	11.39	11.38	9.59	9.57
[Ni ₂ L ₁ X ₂]Y ₂	C ₅₆ H ₄₂ Br ₂ Ni ₂ N ₁₀ O ₆	Brown	1228.2	70	>200	11.39	11.37	9.55	9.54
[Mn ₂ L ₁ X ₂]Y ₂	C ₅₆ H ₄₂ Br ₂ Mn ₂ N ₁₀ O ₆	Black	1220.7	75	>200	11.47	11.45	9.00	9.01

3.1 Molar conductivity

The high molar conductivities of the complexes in 10⁻³ M DMF solution reveal that they are electrolytic in nature, implying that the acetate anion is not coordinated to the central metal ion.

Table 2 Molar conductance data of the binuclear Schiff base metal complexes

Complexes	Solvent	Molar conductance ohm ⁻¹ cm ² mol ⁻¹	Type of electrolyte
[Cu ₂ L ₁ X ₂]Y ₂	DMF	140	1:2
[Co ₂ L ₁ X ₂]Y ₂	DMF	146	1:2
[Ni ₂ L ₁ X ₂]Y ₂	DMF	150	1:2
[Mn ₂ L ₁ X ₂]Y ₂	DMF	141	1:2

3.2 Infrared spectra

A comparison of the spectra of the metal complexes and Schiff bases (Tables 3) indicates that in all the complexes the metal ions are coordinated to the Schiff bases through the imino nitrogen(s) due to the downfield shift in the stretching band of the C=N group on going from ligands (1618 cm⁻¹) to complexes (1600 ± 1602 cm⁻¹). This may be explained based on a drift of the lone pair density of the azomethine nitrogen towards the metal atom (9). New vibrations at 526-572 cm⁻¹ and 447-456 cm⁻¹ which are not present in the free Schiff base are attributed to the existence of ν (M-O) and ν (M-N). The band due to the intramolecular hydrogen bonded OH, appearing in the region 3362 cm⁻¹ in the ligands disappears in the complexes, and the high intensity band of the ligands around 1274 cm⁻¹ due to phenolic ν (C-O) vibration appears in the vicinity of 1299-1301 cm⁻¹ (10,11). These observations emphasize that the -OH groups of the ligands have reacted

with metal(II) via deprotonation.

The band at 1453 cm⁻¹ and 1510 cm⁻¹ were due to symmetric stretching frequency and asymmetric frequency of acetate ion. This result predicts that the acetate ions were coordinated outside the coordination sphere. The spectroscopic behavior of metal complexes of pyridine, shows that after complexation the ring deformation found at 526 - 572 cm⁻¹ and 447- 456 cm⁻¹ in the free pyridine is shifted to higher frequencies (12), indicating coordination via the pyridine nitrogen, as previously reported for pyridine complexes (13). Therefore, this shift is very useful in establishing the participation of pyridine in complex formation.

Table 3 Infrared spectroscopic data of the Schiff base ligand and its binuclear metal complexes

Compounds	Free-OH	C=N cm ⁻¹	Pyridine ring deformations	C-O cm ⁻¹	M-O cm ⁻¹	M-N cm ⁻¹
L ₁	3362	1618	570, 410	1274	-	-
[Cu ₂ L ₁ X ₂]Y ₂	-	1600	621, 420	1299	568	456
[Co ₂ L ₁ X ₂]Y ₂	-	1602	654, 443	1301	570	453
[Ni ₂ L ₁ X ₂]Y ₂	-	1601	653, 423	1300	572	447
[Mn ₂ L ₁ X ₂]Y ₂	-	1600	627, 418	1300	526	456

3.3 Electronic spectra

The bathochromic/ hypsochromic shift from ligand band indicates the complex formation. The two band maxima of ligand are observed between 395 nm and 287 nm due to n-π*, π-π* and charge transfer transitions respectively (14). On the other hand the band corresponding to azomethine showed a slight shift to longer wavelength on going from ligand to complex, indicating coordination of ligand to metals through the azomethine moiety (15,16). The Electronic spectra of Cu(II) complex display one prominent band around 720 nm which is assignable to ²T_{2g} ← ²E_g transition in favour of octahedral structure in DMF medium (17). The binuclear Schiff base Ni(II) complexes exhibit a bands at 265 nm, 380 nm and 530 nm as well as two bands at 630 nm and 720 nm which are attributed to the ³A_{2g}→³T_{2g}(F) and ³A_{2g}→³T_{1g}(P) transitions respectively indicating octahedral geometry around Ni(II) ion. The bands at 520 nm and 660 nm due to the transition ⁶A_{1g}→⁴T_{2g} (G) and ⁶A_{1g}→⁴E_g (G) suggest the high spin octahedral geometry around Mn(II) ion (18). In the present investigation, brownish Co(II) complex show the absorption band at 542 nm, this may be attributed to ⁴T_{1g} (F) →⁴T_{2g} (F) transitions (19).

Table 4 Electronic Spectral data of Schiff base ligand and its binuclear metal complexes.

Compounds	Electronic spectra (nm)				Geometry of the complex
	π→π*	n→π*	L→M	d-d	
L ₁	287	395	-	-	-
[Cu ₂ L ₁ X ₂]Y ₂	290	365	510	720	Octahedral
[Co ₂ L ₁ X ₂]Y ₂	230	360	475	750	
[Ni ₂ L ₁ X ₂]Y ₂	265	380	530	630,720	
[Mn ₂ L ₁ X ₂]Y ₂	250	340	470	520,660	

3.4 Magnetic Moment

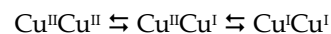
Octahedral cobalt(II) complexes however exhibit 4.82 B.M. (20) show paramagnetic with three unpaired electrons indicating a high-spin octahedral configuration. The magnetic moment of Cu(II) complex was seen at 1.65 B.M. corresponding to one unpaired electron and shows the absence of spin-spin interactions. The magnetic moment of Ni-complex was seen within the range of 2.91 B.M for octahedral Ni(II) complexes (21). The Mn(II) complex show 5.9 B.M. at room temperature corresponding to five unpaired electrons which suggest octahedral geometry (22).

3.5 ¹H NMR spectra

The ¹H NMR Schiff base was recorded in CDCl₃ at room temperature. Three different type of protons were identified i) resonance exhibits due to phenolic -OH protons around 10.21 ppm (23), ii) characteristic resonance due to azomethine proton in the Schiff base appears at 8.126 ppm was observed and iii) the other signals in the region 6.66-7.73 ppm exhibits due to aromatic protons. All these observations support the infrared conclusions.

3.6 Cyclic Voltammogram

All the complexes show two reduction waves at different potentials in the forward scan. In the present binuclear copper(II) complexes, it is very well clear that the binuclear copper complexes undergo two one-electron reductions as follows:



In each redox couple the Ipc/Ipa clearly indicates one electron transfer for redox process. The E_{1/2} values for the first and second redox couples, E_{11/2} and E_{21/2}, fall in the range +0.5 V and -1.35 V respectively. We could expect the above redox process to be metal centred (24). The electrochemical data is thus found to confirm the proposed binuclear structures of the prepared complexes.

The nickel(II) complexes undergo both reduction and

oxidation in cathodic and anodic potentials, respectively. The electrochemical data are summarized in Table 5 and 6. The Reduction processes at cathodic potential were recorded in the potential range 0 to -1.80 V. It is observed that all the binuclear complexes shows two reversible reduction waves in the cathodic potential region and observed that each couple corresponds to one-electron transfer process. The two reduction processes are assigned as follows:



The first reduction potential ranges from -1.0 V, and the second reduction potential lies in the range -0.3 V. Similarly two reduction waves are observed in anodic potential region. It is of interest to compare the electrochemical behavior of the complexes heedfully. The Oxidation process of nickel(II) complexes at anodic potential show two oxidation processes in the range of 0.4-0.9 V. Controlled potential electrolysis experiment indicates that the two oxidation peaks are associated with stepwise oxidation process at nickel(II) center. The data of cyclic voltammograms of the complexes are summarized in Table 6.

The data of cyclic voltammogram of binuclear Mn(II) and Co(II) complexes are tabulated in table 5 and 6. The ΔE_p values suggest the existence of quasireversible couple. The $E_{1/2}$ values indicate that each couple corresponds to one electron transfer process (25).

Table 5 Electrochemical data of binuclear Schiff base metal complexes in DMF medium (reduction)

Complexes	E_{pc} (V)	E_{pa} (V)	$E_{1/2}$ (V)	ΔE (mV)
[Cu ₂ L ₁ X ₂]Y ₂	-1.4	-1.3	-1.35	100
	-0.6	-0.4	-0.5	200
[Co ₂ L ₁ X ₂]Y ₂	-1.2	-1.5	-1.35	300
	-0.7	-0.6	-0.65	100
[Ni ₂ L ₁ X ₂]Y ₂	-1.0	-0.8	-0.9	200
	-0.3	-0.1	-0.2	200
[Mn ₂ L ₁ X ₂]Y ₂	-1.8	-1.7	-1.75	100
	-0.3	-0.6	-0.45	300

Table 6 Electrochemical data of binuclear Schiff base metal complexes in DMF medium (oxidation)

Complexes	E_{pc} (V)	E_{pa} (V)	$E_{1/2}$ (V)	ΔE (mV)
[Cu ₂ L ₁ X ₂]Y ₂	0.7	0.3	0.5	400
	1.1	1.0	1.05	100
[Co ₂ L ₁ X ₂]Y ₂	0.3	0.4	0.35	100
	1.1	0.9	1.0	100
[Ni ₂ L ₁ X ₂]Y ₂	0.6	0.4	0.5	200
	1.0	0.9	1.0	100
[Mn ₂ L ₁ X ₂]Y ₂	0.7	0.6	0.65	100

1.0 1.1 1.05 100

3.7 ESR Spectra

The ESR spectral study provides information of the metal ion environment. The spectrum of the synthesized Cu(II) complex showed bands with $g_{||} > g_{\perp} > g_e$, indicating that the unpaired electrons lay predominantly in the dx^2-dy^2 orbital (26). In the present case 'G' was determined as $G = (g_{||} - 2) / (g_{\perp} - 2)$, which is more than 4 suggesting that there is no interaction between the copper centers (27). Thus, the results suggest that Cu(II) complex possesses distorted octahedral geometry.

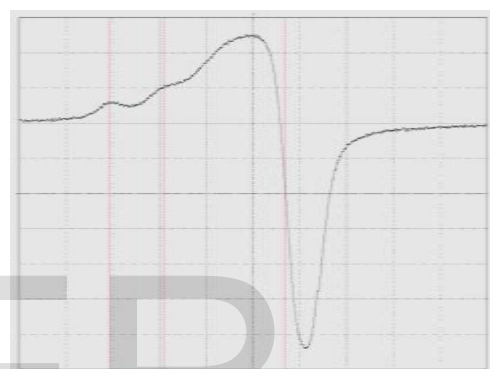


Fig. 4 ESR Spectra of Binuclear Schiff base Cu(II) complex

3.8 Pharmacology results

3.8.1 Antibacterial screening

The antibacterial screenings of the free ligand and the complexes were tested for their effect on certain bacteria. Current studies reveal that higher electronegativity and large atomic radius decreases the effective positive charges on the metal complex molecules and it results to higher antimicrobial activity (28-31).

From fig.5-8, the zone of inhibition area shows to be much larger for metal chelates than that for the ligand but lesser than standard. Such increased activity of the metal chelates can be explained on the basis of chelation theory (32). On chelation, the polarity of the metal ion will be reduced to a great extent due to the overlap of the ligand orbital. Further, it increases the delocalization of p-electrons over the whole chelate ring and enhances the lipophilicity of the complexes (33). This increased lipophilicity leads to breakdown of the permeability barrier of the cell and thus retards the normal cell processes (34,35).

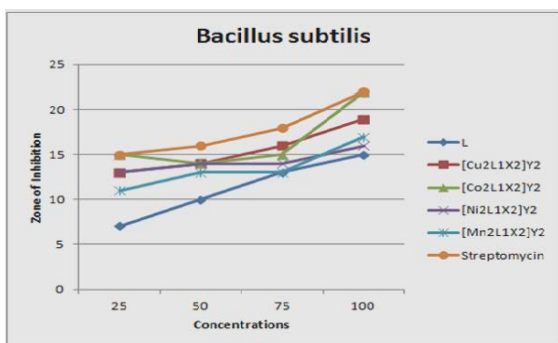


Fig. 5 Antibacterial activity of Schiff base ligand and its binuclear Schiff base metal complexes against *Bacillus subtilis*

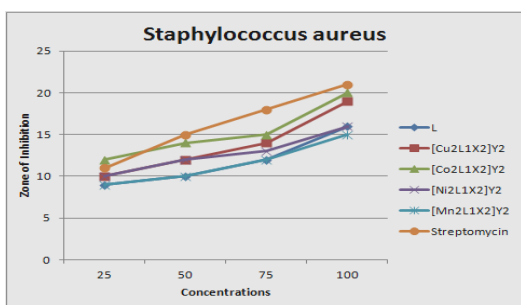


Fig. 6 Antibacterial activity of Schiff base ligand and its binuclear Schiff base metal complexes against *Staphylococcus aureus*

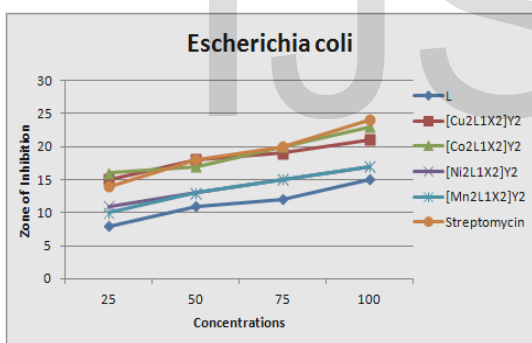


Fig. 7 Antibacterial activity of Schiff base ligand and its binuclear Schiff base metal complexes against *Escherichia coli*

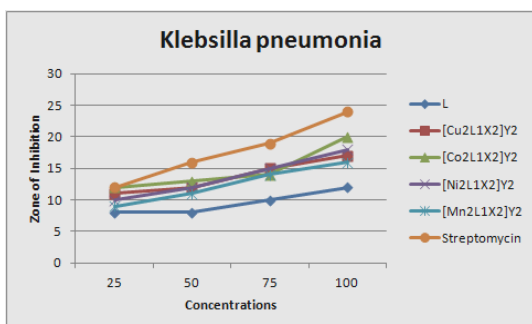


Fig. 8 Antibacterial activity of Schiff base ligand and its binuclear Schiff base metal complexes against *Klebsilla pneumonia*

3.8.2 DNA binding studies

3.8.2.1 Viscosity measurements

The mode of interaction nature between the compounds and DNA can be elucidated through viscosity measurements. In the absence of crystallographic structure data, hydrodynamic methods which are sensitive to DNA length change are regarded as the least ambiguous and the most critical tests of binding in solution. Titrations were performed for the complexes and ligand (0.5–3 μM), and each compound was introduced into DNA solutions (1 μM) present in the viscometer. The plots of $(\eta/\eta_0)^{1/3}$ versus $[\text{Complex}]/[\text{DNA}] = R$, where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solution corrected from the flow time of buffer alone (t_0), $\eta = t - t_0$ gives a measure of the viscosity changes (Fig. 9). A classical intercalation model results in lengthening the DNA helix as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. In addition, some complexes interact with DNA by an electrostatic binding mode; have no influence on DNA viscosity. With an increasing amount of compound, the relative viscosity of DNA increased steadily, which suggests that the complexes can bind to DNA by classical intercalation (36-38). On the basis of all the spectroscopic studies together with the viscosity measurements, we find that the binuclear Schiff base metal complexes can bind to DNA via an intercalative mode.

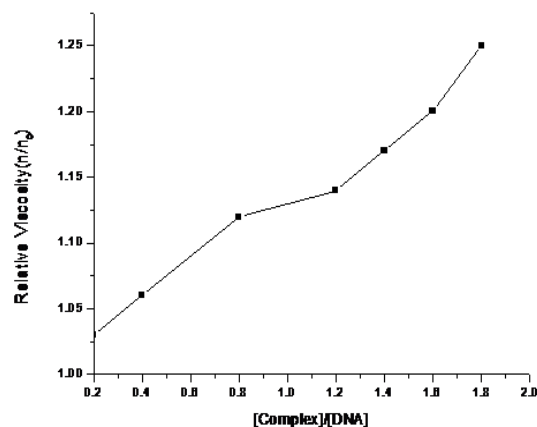


Fig. 9 Viscosity measurements: The effect of the increasing amount of $[\text{Cu}_2\text{L}_1\text{X}_2]\text{Y}_2$ complex on the relative viscosity of DNA at $27 \pm 0.1^\circ\text{C}$ (20 μM, 40 μM, 60 μM, 80 μM, 100 μM).

3.8.2.2 Absorption spectroscopy

A complex bound to DNA through intercalation is characterized by the change in absorbance (hypochromism) and red-shift in wavelength. This may be due to the π^* orbital of the intercalated ligand can couple with the π orbital of the DNA base pairs (stacking interaction between the aromatic

chromophore and the DNA base pairs) thus, decreasing the $\pi \rightarrow \pi^*$ transition energy and resulting in the bathochromism (39). The electronic spectra of the complexes in the presence and absence of DNA were monitored at a wavelength range of 250–600 nm, as shown in Fig.10. The electronic absorption spectra of all the complexes exhibited broad absorption bands in the region 390–400 nm. Upon increasing the concentration of CT DNA (0– 350 μ M), a considerable shift in the wavelength (bathochromicity) was observed for all the complexes. This bathochromicity (red shift) exhibits due to intercalative binding nature of the complexes with the base pair of DNA. The results indicate that the binding strength of complexes are increasing in the order of Cu(II) > Ni(II) > Co(II) > Mn(II).

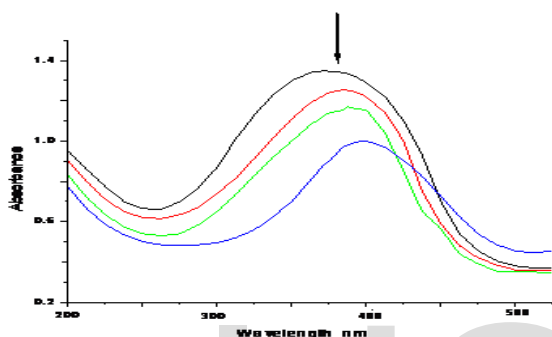


Fig. 10 Absorption spectra of copper(II) complex in the absence and presence of increasing amounts of CT-DNA at room temperature in 50 mM Tris-HCl/ NaCl buffer (pH = 7.2).

Table 7 Absorption properties of Binuclear Schiff complexes-DNA Binding Activity

Complexes	λ_{max} (nm)		$\Delta\lambda$ (nm)
	Free	Bound	
[Cu ₂ L ₁ X ₂]Y ₂	393	399	6.0
[Co ₂ L ₁ X ₂]Y ₂	396	400	4.0
[Ni ₂ L ₁ X ₂]Y ₂	393	398	5.0
[Mn ₂ L ₁ X ₂]Y ₂	397	401	4.0

3.8.3 DNA cleavage activity studies

Deoxyribonucleic acid (DNA) is the principle target molecule for most of the anticancer and antiviral therapies. Gel electrophoresis works on the migration of DNA under the influence of electric potential. The Co(II), Ni(II), Cu(II) and Mn(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method against pUC18DNA and the gel picture showing DNA cleavage in Fig. 11.

The gel electrophoresis clearly revealed that, all the complexes (lanes 1–6) have acted on DNA as there was molecular weight difference between the control and the treated DNA samples. As the complexes were observed to cleave the DNA, it can be concluded that, the complexes inhibits the growth of pathogenic organism by cleaving the

genome (40). Double-strand cleavage of DNA was observed for the copper(II) & Cobalt(II) complexes indicating the present Cu(II) & Co(II) complexes plays the key role in the cleavage (41).

From Fig.11 it is evident that copper complexes cleave DNA more effectively in the presence of oxidant which may be due to hydroxyl radical (OH[•]) reaction with DNA. This is consistent with the increased production of hydroxyl radicals by cuprous ions similar to the well known Fenton reaction. The different DNA-cleavage efficiency of the complexes was due to the different binding affinity of the metal complexes to DNA, which has been observed in other cases (42,43). From these results, we infer that the Co(II), Ni(II), Mn(II) and Cu(II) complexes act as a potent nuclease agents.

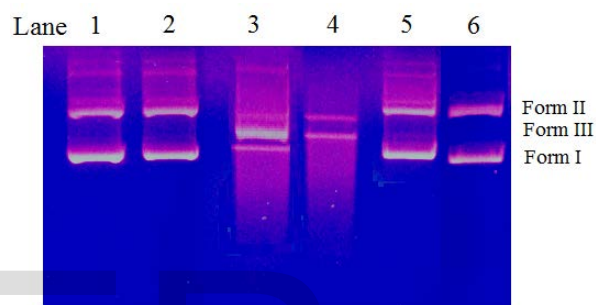


Fig. 11 Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H₂O₂ and metal complexes. From left to right: Lane 1-DNA alone; Lane 2- DNA alone + H₂O₂; Lane 3-DNA + [Cu₂L₁X₂]Y₂ + H₂O₂; Lane 4-DNA + [Co₂L₁X₂]Y₂ + H₂O₂; Lane 5 -DNA + [Ni₂L₁X₂]Y₂ + H₂O₂; Lane 6-DNA+ [Mn₂L₁X₂]Y₂ + H₂O₂.

4 Conclusions

In this paper, we have explored the synthesis and coordination chemistry of some binuclear complexes derived from the octadentate Schiff-base ligand H₂L and bidentate ligand. The ligand behaves as a dibasic octadentate species upon complexation with the involvement of phenolic oxygen and the nitrogen atoms of the pyridyl groups in coordination for all the complexes. The newly synthesized Schiff base and its metal complexes have been confirmed by the analytical data, IR, UV-Visible, ¹H NMR, ESR spectral data, magnetic moment, molar conductance and thermal studies. The antibacterial activity results shows that all complexes have been found to be more potent than its ligand due to the process of chelation dominantly affects the overall biological behavior of the complexes. The DNA cleavage studies revealed that the metal complexes showed good efficiency towards DNA cleavage. Based on the analytical and spectral studies, we propose dimeric octahedral geometry to all the synthesized complexes (Fig. 2).

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